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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

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For: FUNGICIDAL AND BACTERICIDAL COMPOSITIONS FOR PLANTS CONTAINING PHOSPHONATE AND PHOSPHATE SALTS, METAL CHELATES AND DERIVATIVES THEREOF

Inventor: Taylor, John B. — Date: 18 June 2003

DECLARATION OF DR. JOHN B. TAYLOR

I, ~~Dr.~~ John B. Taylor, declare as follows:

1. I am named as an inventor on several United States Patents relating to fungicides and related technology. These include:

6,509,041	Compositions for plants containing phosphonate and phosphate salts, and derivatives thereof.
6,338,860	Compositions for plants containing phosphonate and phosphate salts, and derivatives thereof.
6,139,879	Fungicidal and bactericidal compositions for plants containing compounds in the form of heavy metal chelates.
5,997,910	Plant fertilizer compositions containing phosphonate and phosphate salts and derivatives thereof.
5,925,383	Fungicidal compositions for plants containing phosphonate and phosphate salts, and derivatives thereof.
5,800,837	Plant fertilizer compositions containing phosphonate and phosphate salts and derivatives thereof.
5,736,164	Fungicidal compositions for plants containing phosphonate and

phosphate salts, and d rivatives thereof.

2. I am named as the sole inventor of th subject matter d scribed and claimed in this application.

3. In January 2003, I initiated a cooperative test to evaluate the CuEDDHA/PO3 + PO4 combination in the control of *Xanthomonas campestris pv campestris* in the systemic phase, which is exceedingly difficult to control. This disease is "Black Rot" of crucifers (cabbage, cauliflower, etc.) and is one of the most difficult bacterial pathogens to manage in the production of these crops.

4. My collaborator in this effort was Dr. James O. Strandberg, Professor of Plant Pathology at the University of Florida, Institute of Food and Agricultural Sciences, Mid-Florida Research and Education Center, Apoka Florida. Dr. Strandberg is one of the nation's leading experts in plant pathology.

5. My job in this effort was to prepare the experimental solutions and spray them on the plants. Cabbage was chosen as the test plant. Dr. Strandberg's job was to inoculate the sprayed plants with *Xanthomonas campestris pv campestris* and observe and record the results.

6. I prepared three experimental solutions:

- FNX-100, a solution of PO3/PO4 @ 2% v/v.
- FNX-200, a solution of CuEDDHA @ 0.2 lb. Cu ai/100 gal.
- FNX-2000, a solution of FNX-100 @ 2% + FNX-200 0.2lbs ai Cu.

7. The cauliflower plants were sprayed by me one week prior to

inoculation by Dr. Strandberg. Two weeks after inoculati n, percent systemic infection evaluations were mad by Dr. Strandberg. He prepared a report f his results, a copy of which is attached is Exhibit A.

8. In part, the results for percent of plants with systemic infections were as follows

- FNX-100 (PO3/PO4) @ 2% v/v had an infection level of 21.0%.
- FNX-200 (CuEDDHA @ 0.2 lb. Cu al/100 gal) had an infection level of 34.7%.
- FNX-2000 (FNX-100 @ 2% + FNX-200 0.21bs al Cu) had an infection level of 1.39%.

9. The reported results for a disease damage rating are subjective and not reliable.

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EFFECTS OF SOME COPPER FUNGICIDES AND SYSTEMIC ACQUIRED RESISTANCE INDUCING COMPOUNDS ON THE DEVELOPMENT OF *Xanthomonas campestris* pv. *Campestris* ON CAULIFLOWER SEEDLINGS

James O. Strandberg[1]

Plant Pathology Research Report 2003 - 1 January, 20003

SUMMARY: Effects of some copper fungicides and systemic acquired resistance (SAR) inducing materials (neutral salts of phosphorous acid)) on the development of black rot of cauliflower caused by *Xanthomonas campestris* pv *campestris* were compared for possible control or suppression of this disease. Disease incidence and damage was substantial in this experiment following artificial inoculation of cauliflower seedlings growing in the greenhouse. Treatments of copper hydroxide and neutral salts of phosphorus acid alone, or when mixed with chelated copper significantly (P=0.5) reduced the percent of leaf area damaged by black rot.

METHODS: Hybrid cauliflower seeds cv 'Snow Queen' were germinated in vermiculite and transplanted 12 days later into plastic-cell trays containing a peat, sand, vermiculite potting mix. Plants were grown in a warm green house (60 F night 80 F day) and watered by overhead sprinkler irrigation as needed. A 20–20–20 soluble fertilizer was applied once. On December 31, three weeks after transplanting, treatments specified in Table1 were applied with a household spray bottle. FNX-100 and FNX 2000 were provided by Foliar Nutrients, Inc., Valdosta, GA. Seven days after treatments were applied, the plants were placed in a mist chamber and periodically misted (every 15 min) for 6 hours. Plants were inoculated and left in the mist chamber (without misting, but plants remained wet) for an additional 16 hours, then moved to the greenhouse.

To inoculate plants, cells of *X. campestris* pv. *campestris* were grown in nutrient broth in shake culture for 24 hr at 28 C, centrifuged, washed with saline, then re-suspended in saline. Plants were uniformly inoculated with the cell suspension by spraying them with a household spray bottle on January 8, 2003. Plants in the experimental units

[1]Professor of Plant Pathology

were evaluated for leaf spot and yellowing symptoms on January 21 and infected leaves were subjectiv ly rated for percent of total leaf area damaged by the pathogen. The rating system estimated the percent of total leaf area damaged by leaf spots and yellowing (Table 1). On January 26, plants were removed from pots and the proportion showing systemic infection (yellow lesions, necrosis, and blackened veins) was determined. There were six replicates per treatment containing 12 plants each, except for the control treatment which had eight replicates. Data were analyzed by a one-way ANOVA; means were separated with Student-Newman-Keul's method (P 0.05). In analyzing data for the proportion of systemically-infected plants, a one-way ANOVA was run on the ARCSIN transformed percentage values.

RESULTS AND DISCUSSION

About 8 – 10 days after inoculation, abundant leaf spot and yellowing symptoms appeared; apparently most infections occurred through stomates. These symptoms consisted of numerous small, black flecks and spots accompanied by leaf yellowing. Later, systemic infection and large, yellow lesions typical of black rot appeared in most treatments. There were significant differences in the percent of total leaf area infected and damaged by leaf spots and yellowing caused *by X. campestris* among the treatments (Table 1). Several of the treatments reduced leaf spot and yellowing damage compared with the unsprayed control. One week later, there were also significant differences in proportions of plants with typical, systemic-black rot symptoms among the treatments (Table 1). These results demonstrate that SAR compounds such as neutral salts of phosphorous acid and their mixtures with copper can be effective in suppressing disease damage caused by *Xanthomonas* spp. when applied as a preventative treatment to foliage.

Table 1. Effects of some copper fungicides and systemic acquired resistance inducers on the infection and damage by *Xanthomonas campestris* pv. *campestris* on cauliflower seedlings. January, 2003.

Treatment	Application Rate	Disease damage[1] rating	Percent of plants[2] with systemic infections
1. FNX - 100	½ % vol/vol	0.25 a	32.4 bc
2. FNX - 100	1 % vol/vol	1.58. b	43.2 c
3. FNX - 100	2 % vol/vol	1.00 ab	21.0 b
4. FNX - 200	0.2 lb a.i. Cu	0.70 ab	34.7 bc
5. FNX - 200 + FNX - 100	0.2 lb a.i. Cu 2 % vol/vol	2.75 c	1.39 a
6. Kocide 2000	1.5 lb a.i. Cu	0.33 a	18.6 b
7. Control	----------	3.75 d	59.3 d
	F =	24.956	12.355
	P =	<0.00	<0.00

[1] Disease damage index based on estimated percent of total leaf area damaged by *X. campestris* pv. campestris. Damage index X 10 = approximate percent of total leaf area damaged. One-way ANOVA on 6 replicates per treatment except control which had eight replicates. Values followed by the same letter are not significantly different at the *P* = 0.05 level. Mean separation by Student-Newman-Keuls method.

[2] Average percent of plants showing typical systemic infection symptom, leaf necrosis and black veins. One-way ANOVA on ARCSIN transformation of percentage values.. Values followed by the same letter are not significantly different at the *P* = 0.05 level. Mean separation by Student-Newman-Keuls method.